Chapter 14

Nanostructure Imaging Mass Spectrometry: The Role of Fluorocarbons in Metabolite Analysis and Yoctomole Level Sensitivity

Michael E. Kurczy, Trent R. Northen, Sunia A. Trauger, and Gary Siuzdak

Abstract

Nanostructure imaging mass spectrometry (NIMS) has become an effective technology for generating ions in the gas phase, providing high sensitivity and imaging capabilities for small molecules, metabolites, drugs, and drug metabolites. Specifically, laser desorption from the nanostructure surfaces results in efficient energy transfer, low background chemical noise, and the nondestructive release of analyte ions into the gas phase. The modification of nanostructured surfaces with fluorous compounds, either covalent or non-covalent, has played an important role in gaining high efficiency/sensitivity by facilitating analyte desorption from the nonadhesive surfaces, and minimizing the amount of laser energy required. In addition, the hydrophobic fluorinated nanostructure surfaces have aided in concentrating deposited samples into fine micrometer-sized spots, a feature that further facilitates efficient desorption/ionization. These fluorous nanostructured surfaces have opened up NIMS to very broad applications including enzyme activity assays and imaging, providing low background, efficient energy transfer, nondestructive analyte ion generation, super-hydrophobic surfaces, and ultra-high detection sensitivity.

Key words Nanostructure imaging mass spectrometry (NIMS), Desorption/ionization on silicon mass spectrometry (DIOS-MS), Metabolites, Mass spectrometry imaging

1 Introduction

Desorption mass spectrometry has undergone significant advancements since it was first developed more than a century ago [1]. A major improvement occurred in the early 1980s, with the development of matrix-assisted laser desorption/ionization (MALDI), a method of nondestructively transferring laser energy to the analyte by using a light-absorbing organic matrix [2, 3]. However, the use of organic matrices can present interference when attempting to detect small molecules less than 500 Da (e.g., metabolites). Therefore, in 1999 a matrix-free nanostructure imaging mass spectrometry (NIMS) strategy for mass spectrometry was introduced

Lin He (ed.), *Mass Spectrometry Imaging of Small Molecules*, Methods in Molecular Biology, vol. 1203,
DOI 10.1007/978-1-4939-1357-2_14, © Springer Science+Business Media New York 2015

based on using pulsed-laser desorption/ionization with a silicon nanostructured surface [4]. This method, originally called desorption/ionization on silicon mass spectrometry (DIOS-MS), uses laser irradiation to desorb and ionize analytes from a porous silicon surface, eliminating the need for organic matrices and thus extending the measurable mass below 500 Da [4]. Surface modifications of silicon nanostructured surfaces were later found to allow more efficient ion generation and resistance to oxidation [5, 6]. And more recently, the introduction of liquid fluorous compounds onto the nanostructured surface to form clathrates has resulted in improved detection capabilities as well as the ability to perform high-resolution imaging [7–10]. In this chapter, we discuss the possible mechanisms behind nanostructure desorption/ionization and the ultrahigh sensitivity that can be achieved with NIMS.

2 Nanostructure-Based Desorption/Ionization

One of the unique features of the NIMS desorption/ionization approach is its large surface area. High-surface-area porous silicon nanostructures facilitate efficient laser absorption and aid in the desorption/ionization of intact molecular ions through a laser-induced rearrangement of the surface structure [11–14] (Fig. 1). The large surface area (as large as 200 m^2/cm^2) can reduce the melting point of silicon; therefore laser-induced surface restructuring is thought to be the driver for analyte desorption [12]. The process is also highly dependent on the laser energy which directly correlates with ion generation. The low-threshold laser energy required for ion generation (10 mJ/cm^2), when compared to other desorption/ionization techniques like MALDI (40 mJ/cm^2), suggests that desorption/ionization is driven by surface restructuring and is not strictly a thermal process [12]. Similarly, the silicon nanowire [15], silicon nanopost arrays (NAPA) [16], laser-induced silicon microcolumn arrays (LISMA) [17], and other nanostructure-based techniques [14] likely work in a similar fashion; increased surface area typically lowers the laser energy required for analyte desorption.

Hydrophobic fluorous materials introduced into nanostructured surfaces have also played an important role in producing surfaces that allow for improved performance for NIMS including enhanced sensitivity (Fig. 2). Three different methods have been developed to incorporate fluorous compounds within porous silicon nanostructures. First, silicon nanostructures have been designed with a covalent pentafluorophenyl modification to reduce analyte adhesion and protect the porous surface from oxidation [5]. A second method has applied the addition of fluorous surfactants, such as perfluoroundecanoic acid, with the pentafluorophenyl-modified silicon surface. These surfaces have been shown to be more effective at reducing analyte adhesion and

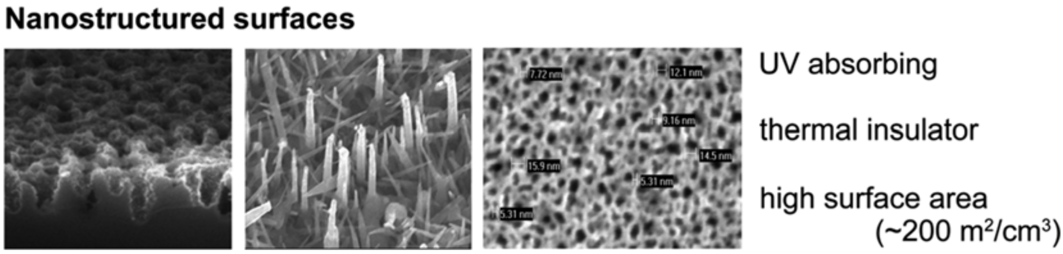

Fig. 1 Electron micrographs of silicon-based nanostructure surfaces used in NIMS experiments. A unique feature of these surfaces is that they are UV-absorbing thermal insulators with a large surface area, facilitating their unique desorption/ionization properties

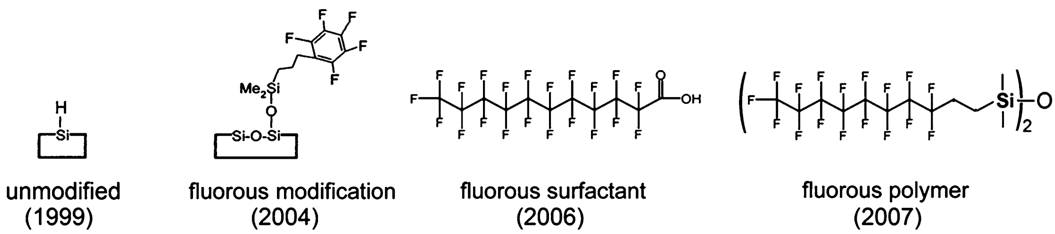

Fig. 2 The evolution of fluorous modifications on the nanostructured surfaces, including unmodified surfaces in 1999 [4], chemical modification in 2004 [5], surfactants in 2006 [6], and teflon-like fluorous polymers such as bis(heptadecafluoro-1,1,2,2-tetrahydrodecyl)tetramethyl-disiloxane in 2007 [7]

improving desorption/ionization efficiency [6]. The third method, introduced in 2007, employs fluorous siloxanes as liquid initiators to coat the porous silicon nanostructure surface and further minimize analyte adhesion [7]. With this NIMS technology, it was found that fluorous siloxane initiators did not absorb laser light or ionize, and therefore do not contribute chemical noise in the spectrum, a very important aspect of the NIMS design. Subsequent laser-induced heating transfers energy to the trapped liquid phase, causing rapid initiator vaporization and desorption/ionization of the intact analytes without fragmentation. Among its features is that this surface is stable in ambient air, has an expanded mass range, and can be used to analyze biofluids and image tissues (Fig. 3). The versatility of the fluorinated NIMS platform has now been demonstrated for a large variety of analytes, ranging from metabolites and drugs to peptides and proteins [4–7].

3 Ultrahigh-Sensitivity Detection

The ultrahigh sensitivity that can be obtained with NIMS has been successfully demonstrated with specific analytes down to the yoctomole level as shown in Fig. 4. The first report of yoctomole sensitivity with NIMS was using a pentafluorophenyl-silylated

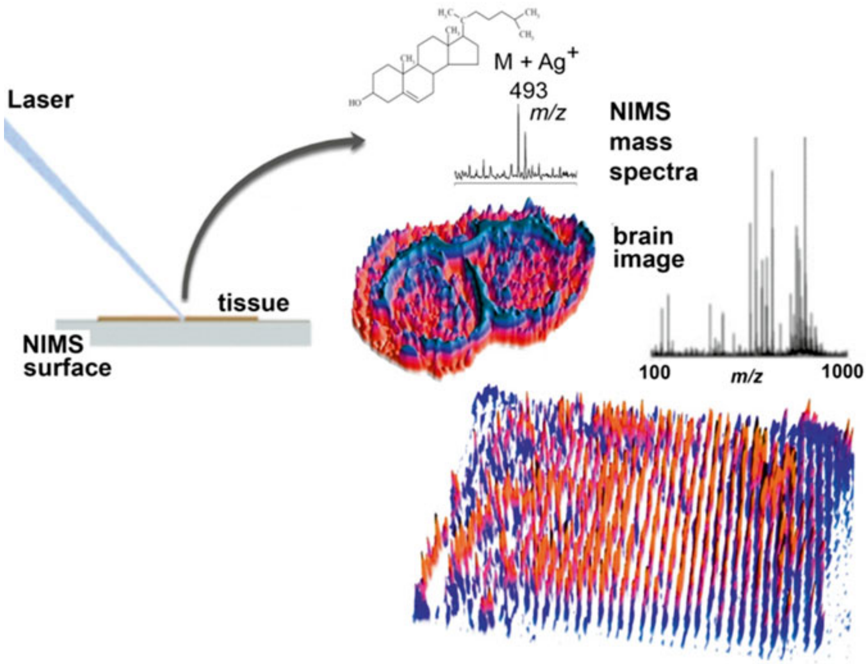

Fig. 3 Nanostructure imaging mass spectrometry (NIMS) of a brain tissue and also imaging of a plate containing 1,500 discrete chemical entities spotted on the NIMS surface

nanostructure silicon surface to analyze des-Arg9-bradykinin (des-Arg9-bradykinin is commonly used by instrument manufacturers to test sensitivity). Here a series of dilution experiments was carried out to ultimately demonstrate a lower limit of detection for the peptide at 480 molecules (800 ymol) (Fig. 3a) [5]. Similarly, NIMS was also found to have yoctomole detection for small molecules where lower limits of detection of 700 ymol for verapamil [18] and 650 ymol for propafenone have been observed [19] (Fig. 3b). Given the significance of this unprecedented sensitivity, the experiments were replicated on ten separate occasions by three different individuals.

4 Mechanistic Discussions

An important question to consider is why NIMS is inherently more sensitive than traditional matrix-assisted approaches such as MALDI, especially given that these experiments are performed with the same instrumentation. While very impressive, MALDI with high sensitivity (low zeptomole) has been achieved by Keller and Li [20], MALDI however is typically 50 times less sensitive than NIMS. To assess this difference in sensitivity, sample deposition was initially examined as this is a key feature that differs

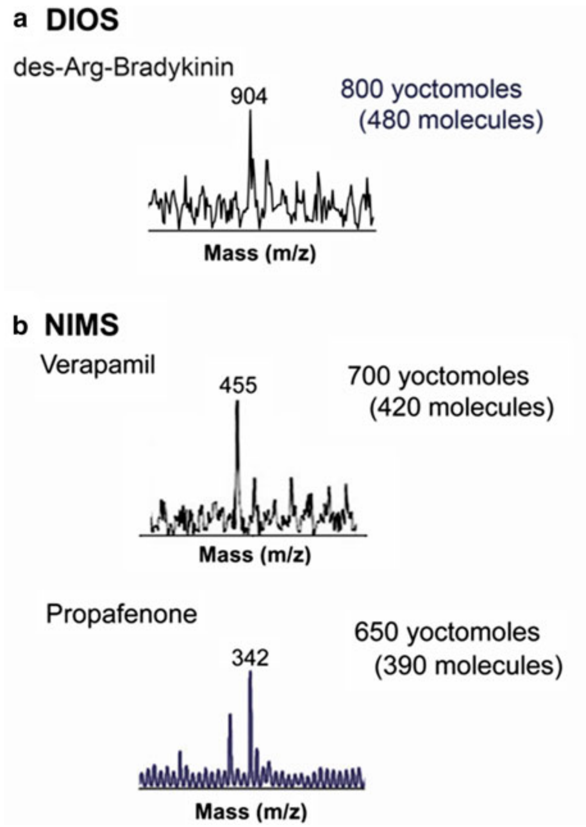

Fig. 4 High-sensitivity nanostructure imaging mass spectrometry (NIMS) experiments. Detection limit of (**a**) 480 molecules (800 ymol) for des-Arg9-bradykinin using pentafluorophenyl-functionalized porous silicon and (**b**) 420 molecules (700 ymol) for verapamil and 390 molecules (650 ymol) of propafenone using a bis(tridecafluoro-1,1,2,2-tetrahydrooctyl) tetramethyldisiloxane initiator

between NIMS and MALDI. In typical NIMS experiments the sample droplet is spotted directly onto the nanostructured surface. The unique nonadhesive surface properties of the fluorinated modifications and coatings used for NIMS not only reduce adhesion of the analyte facilitating desorption, but also the hydrophobic nature of the coating results in the formation of small aqueous droplets that concentrate the analyte on the surface. Simply put, the aqueous droplet being hydrophilic minimizes its contact area with the fluorinated coating and dries in a smaller spot concentrating the analyte. Another advantage of this technique is in its application to real biological samples and biofluids, which often contain salts and buffers which are detrimental to mass spectrometry. The process of analyte concentration on the hydrophobic fluorinated coating separates the salts to the outer edges, essentially "cleaning up" the analyte for analysis. The hydrophobic-hydrophobic interaction occurring between the fluorocarbon and the analyte serves to corral these molecules on the nanostructured surface, minimizing the number of analyte molecules in a given area necessary to produce the analyte signal. In many cases it is possible to adsorb analyte onto the fluorous surface directly from the sample droplet to minimize the effects of interferences within the sample (e.g., salts, proteins). This is again thought to be a result of the high surface energy at the fluorous-aqueous boundary that drives adsorption of molecules with amphipathic characteristics to the interface. The concentration effect can easily improve the detection sensitivity by a factor of 10–100. This can enable a signal to be generated from a small amount of material that is quickly consumed with a few laser shots.

Another distinguishing feature between nanostructure-based desorption/ionization and MALDI is that MALDI incorporates analytes into the matrix crystals which can affect its sensitivity, as does the ionization of the matrix materials, causing analyte signal suppression. Thus in MALDI, the spatial limitation of analytes exists both laterally across the surface as well as being dependent on the matrix crystal thickness/depth (microns to millimeters in size). The resulting laser-induced ablation following each laser shot introduces new crystal surfaces from which a signal can be produced. The crystal thickness allows for a continuous signal in MALDI, yet it also introduces a dilution effect of the analyte in the matrix crystal. This dilution effect, while effective in providing a signal that continues over many laser pulses, is ultimately detrimental to achieving the highest level of sensitivity.

The length of signal duration is also quite different between nanostructure-based laser desorption/ionization and MALDI. Typically NIMS generates a signal for a significantly shorter number of laser pulses (3–50) whereas MALDI can generate a signal for hundreds if not thousands of laser shots before signal depletion occurs. The shorter signal duration characteristic of the nanostructured surfaces is likely due to the very different nature of the matrix-free nanostructure versus matrix-induced events that can occur by using MALDI. Since NIMS [14] are surface-induced phenomena, the generation of a signal is largely a 2D surface phenomenon versus 3D matrix crystals that depends on the nanosecond duration of the thermal and surface-restructuring events. Having a signal from a larger packet of ions in fewer laser shots provides a higher signal/noise ratio (S/N) since it contains a fixed amount of noise. When data is averaged over a larger number of spectra, the S/N only increases in proportion to the square root of the number of shots taken and the relatively low surface concentration in NIMS is quickly depleted. Therefore, averaging spectra from multiple laser shots ultimately results in a lower S/N than getting a larger burst of ions detected. This is analogous to LC-MS where increasing chromatographic resolution with techniques like UPLC or smaller ID columns like nano and capillary LC boosts sensitivity. Finally, an additional difference observed is in the laser energy used in NIMS (~10 mJ/cm^2) which is significantly lower than that used for MALDI (~40 mJ/cm^2 or higher). These lower energies can presumably reduce extraneous signal that can occur as a result of fragmentation of analyte, thereby minimizing the accumulation of background noise and improving the S/N.

5 Conclusion

The high detection sensitivity that can be obtained by using NIMS is the result of efficient ion generation from these surfaces as well as extremely low background noise. As discussed, modifying the surface with fluorous compounds is very important to achieve yoctomole sensitivity. In addition, engineering the nanostructures could further enhance the detection sensitivity. For example, Vertes et al. demonstrated that nanofabrication of ordered monolithic silicon nanostructures such as NAPA, with optimized array geometries (including height and diameter of nanopost and post-to-post distance), has the potential to improve the detection sensitivity [14, 21]. Theorizing that the optimization of the array geometries enhances the nanostructure-laser interaction, therefore improving ion production; NAPA was capable of detecting ~800 zmol of verapamil [21]. Therefore the combination of ordered nanostructured surfaces with fluorous surface modifications could further improve detection sensitivity beyond what has been observed thus far.

Currently, manual deposition is the most commonly used approach for sample deposition in nanostructure-based desorption/ionization MS experiments. In these cases deposition quantities typically range from 0.1 to 0.5 μl of sample solution. Alternatively, the use of more accurate sample deposition techniques (e.g., acoustic deposition) that effectively reduce the deposited sample volume could concentrate the sample to an even smaller spot size, and improve detection sensitivity. Acoustic deposition is capable of precisely depositing ~100 pl sized droplets onto a surface with spot size as low as 60 μm [22]. Therefore, the combination of ultra-fine sample deposition techniques with the concentrating effect of a hydrophobic nanostructured surface may provide another possible way to further improve the sensitivity.

The biological implications of ultrahigh detection sensitivity are especially significant given its potential application to single-cell analysis [23, 24]. One significant application would be the ability to observe single-cell heterogeneity and elucidate the role that each cell plays in the function of a biological system. The size of a cell is typically 1–100 μm, with a volume of ~30 fl. With the concentrations of major metabolites in cells in the attomole range [14], nanostructure-based desorption/ionization mass spectrometry exhibits a limit of detection down to yoctomole level, making metabolic imaging of single cells (i.e., intracellular metabolite biodistributions) possible to explore. Given the importance of ultrahigh detection sensitivity for single-cell analysis, nanostructure-based desorption/ionization mass spectrometry could ultimately play an important role in these analyses, providing new insights into cellular biology.

Acknowledgments

This work conducted by ENIGMA-Ecosystems and Networks Integrated with Genes and Molecular Assemblies was supported by the Office of Science, Office of Biological and Environmental Research, of the US Department of Energy under Contract No. DE-AC02-05CH11231. This work was also supported by the California Institute of Regenerative Medicine Grant TR1-01219 and the National Institutes of Health grants R24 EY017540-04, P30 MH062261-10, and P01 DA026146-02. Financial support was also received from the Department of Energy grants FG02-07ER64325 and DE-AC0205CH11231.

References

1. Thomson JJ (1910) Rays of positive electricity. Phil Mag 20:752–767
2. Karas M, Bachmann D, Bahr U, Hillenkamp F (1987) Matrix-assisted ultraviolet laser desorption of non-volatile compounds. Int J Mass Spectrom Ion Process 78:53–68
3. Karas M, Hillenkamp F (1988) Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons. Anal Chem 60:2299–2301
4. Wei J, Buriak JM, Siuzdak G (1999) Desorption-ionization mass spectrometry on porous silicon. Nature 399:243–246
5. Trauger SA, Go EP, Shen ZX, Apon JV, Compton BJ, Bouvier ESP, Finn MG, Siuzdak G (2004) High sensitivity and analyte capture with desorption/ionization mass spectrometry on silylated porous silicon. Anal Chem 76: 4484–4489
6. Nordstrom A, Apon JV, Uritboonthal W, Go EP, Siuzdak G (2006) Surfactant-enhanced desorption/ionization on silicon mass spectrometry. Anal Chem 78:272–278
7. Northen TR, Yanes O, Northen MT, Marrinucci D, Uritboonthai W, Apon J, Golledge SL, Nordstrom A, Siuzdak G (2007) Clathrate nanostructures for mass spectrometry. Nature 449:1033–1036
8. Yanes O, Woo HK, Northen TR, Oppenheimer SR, Shriver L, Apon J, Estrada MN, Potchoiba MJ, Steenwyk R, Manchester M, Siuzdak G (2009) Nanostructure initiator mass spectrometry: tissue imaging and direct biofluid analysis. Anal Chem 81:2969–2975
9. Patti GJ, Woo HK, Yanes O, Shriver L, Thomas D, Uritboonthai W, Apon JV, Steenwyk R, Manchester M, Siuzdak G (2010) Detection of carbohydrates and steroids by cation-enhanced nanostructure-initiator mass spectrometry (NIMS) for biofluid analysis and tissue imaging. Anal Chem 82:121–128
10. Greving MP, Patti GJ, Siuzdak G (2011) Nanostructure-initiator mass spectrometry metabolite analysis and imaging. Anal Chem 83:2–7
11. Kruse RA, Li X, Bohn PW, Sweedler JV (2001) Experimental factors controlling analyte ion generation in laser desorption/ionization mass spectrometry on porous silicon. Anal Chem 73:3639–3645
12. Northen T, Woo H-K, Northen M, Nordström A, Uritboonthail W, Turner K, Siuzdak G

(2007) High surface area of porous silicon drives desorption of intact molecules. J Am Soc Mass Spectrom 18:1945–1949

13. Peterson DS (2007) Matrix-free methods for laser desorption/ionization mass spectrometry. Mass Spectrom Rev 26:19–34
14. Stolee JA, Walker BN, Zorba V, Russo RE, Vertes A (2012) Laser-nanostructure interactions for ion production. Phys Chem Chem Phys 14:8453–8471
15. Go EP, Apon JV, Luo GH, Saghatelian A, Daniels RH, Sahi V, Dubrow R, Cravatt BF, Vertes A, Siuzdak G (2005) Desorption/ionization on silicon nanowires. Anal Chem 77:1641–1646
16. Walker BN, Stolee JA, Pickel DL, Retterer ST, Vertes A (2010) Tailored silicon nanopost arrays for resonant nanophotonic ion production. J Phys Chem C 114:4835–4840
17. Chen Y, Vertes A (2006) Adjustable fragmentation in laser desorption/ionization from laser-induced silicon microcolumn arrays. Anal Chem 78:5835–5844
18. Two different individuals (S. Trauger and J. Apon) performed the experiments independently.
19. Two different individuals (W. Uritboonthai and O. Yanes) performed the experiments independently.
20. Keller BO, Li L (2001) Detection of 25,000 molecules of substance P by MALDI-TOF mass spectrometry and investigations into the fundamental limits of detection in MALDI. J Am Soc Mass Spectrom 12:1055–1063
21. Walker BN, Stolee JA, Vertes A (2012) Nanophotonic ionization for ultratrace and single-cell analysis by mass spectrometry. Anal Chem 84:7756–7762
22. Aerni H-R, Cornett DS, Caprioli RM (2005) Automated acoustic matrix deposition for MALDI sample preparation. Anal Chem 78: 827–834
23. Trouillon R, Passarelli MK, Wang J, Kurczy ME, Ewing AG (2012) Chemical analysis of single cells. Anal Chem 85:522–542
24. O'Brien PJ, Lee M, Spilker ME, Zhang C, Yan Z, Nicholls TC, Li W, Johnson CH, Patti GJ, Siuzdak G (2013) Monitoring metabolic responses to chemotherapy in single cells and tumors using nanostructure-initiator mass spectrometry (NIMS) imaging. Cancer Metab 1:4